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CISSUS QUADRANGULARIS ASSISTED BIOSYNTHESIS OF SILVER NANOPARTICLES WITH ANTIMICROBIAL AND ANTICANCER POTENTIALS

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ABSTRACT

Using biological medium, such as plant extract for the biosynthesis of nanoparticles is an ecofriendly and emerging scientific trend. On this back drop the present study focused on the biosynthesis of silver nanoparticles using *Cissus quadrangularis* and evaluation of its antimicrobial and anticancer potentials. The biosynthesized nanoparticle was characterized by UV-VIS spectroscopy, EDAX and TEM. UV–visible spectrum of the aqueous medium containing silver nanoparticles showed a peak around 422 nm. The TEM and EDAX analysis showed that the particles were silver nanoparticles in the range of 5-30nm. The antimicrobial activity of the nanoparticles was studied against Gram positive and Gram negative bacteria. The cytotoxicity of the nanoparticles was studied against Hep2 and Vero cell line and the IC 50 value was found to be 64µg and 90 µg for Hep2 and Vero cell line respectively.

KEYWORDS : Antimicrobial activity, anticancer activity, Biosynthesis, Silver nanoparticles, *Cissus quadrangularis,* Characterization.

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INTRODUCTION

Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and their potential use for human benefits [1]. Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Nanoparticles have been studied extensively because of their unique physicochemical characteristics including catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties [2].

In recent years, plant- mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and ecofriendliness. The reducing property of different plant constituents may play a critical role in the reduction of Ag^+ to silver nanoparticles. Biosynthesis of gold and silver nanoparticles by plants such as *Alfalfa* [3], *Aloe vera* [4], *Cinnamomum camphora* [5], *Emblica officianalis* [6], *Carica papaya* [7], *Parthenium hysterophorus* [8], *Azadirachta indica* [9], *Eucalyptus hybrid* [10], *Hibiscus rosasinensis* [11], *Capsicum annuum* [12], *Boswellia ovalifoliolata* [13] and *Azadirachta indica* [14] have been reported. The synthesis of silver nanoparticles using plant constituents has not yet been studied for a large number of natural compounds. In this study, the synthesis of silver nanoparticles using *Cissus quadrangularis* has been investigated and the antimicrobial and cytotoxicity of silver nanoparticles were studied.

MATERIALS AND METHODS

Plant material and preparation of the Extract

Cissus quadrangularis stem was collected from Tamil Nadu, India. Green young stem of *Cissus quadrangularis* (commonly called as pirandai, in Tamil Nadu, India) was used to prepare the aqueous extract. Young stem weighing 25g were thoroughly washed in distilled water, cut into fine pieces and were crushed with 100 ml sterile distilled water to get stem extract. The stem extract was filtered through Whattman No.1 filter paper (42µm) and used for the further procedures.

Synthesis of silver nanoparticles

A modified method of the studies of Saifuddin *et al* (2009) was followed for microwave irradiation method for rapid synthesis of silver nanoparticles [15]. A mixture of 100ml of 1mM silver nitrate solution with 5 ml of plant extract was treated with microwave irradiation till the color changes to yellowish-brown. Color change (brown color change) indicates the reduction of silver nitrate into silver nanoparticles [16].

Characterization of synthesized silver nanoparticles

The synthesized silver nanoparticles were characterized by UV-Vis spectra analysis, TEM and EDAX analysis.

(i) UV-Vis Spectra analysis

The reduction of silver nitrate was observed after 5 min exposure of solution to microwave irradiation. The reduction was visually observed by change in color of the solution. The color was changed from colorless to yellowish brown color. Further the reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium and the absorbance was recorded at 200-800 nm using UV-Vis spectrophotometer (VARIAN CARY EL06023680).

(ii) TEM analysis of silver nanoparticles

Transmission electron microscope was done in TANUVAS, Chennai. By drop coating, silver nanoparticle were prepared for High-resolution transmission electron microscope analysis on to carbon coated copper TEM grids. The film on the TEM grids were allowed to stand for 2 min following which the extra solution was removed using a blotting paper and grid was allowed to dry, prior to the measurement. HR TEM measurements were performed on a

JEOL 3010 instrument operated at an accelerating voltage of 300kV.

(iii) EDAX measurements

In order to carry out EDAX analysis, the extracts reduced silver nanoparticles were dried and drop coated on to carbon film and performed on Hitachi S-3400 NSEM instrument equipped with a Thermo EDAX attachments. Energy dispersive X-ray spectrometers take advantage of the photon nature of light. In the X-ray range the energy of single photon is just sufficient to produce a measurable voltage pulse X-ray, the output of an ultra low noise preamplifier connected to the low noise are a statistical measure of the corresponding quantum energy. By digitally recording and counting a great number of such pulses within a so called multi channel analyze a complete image of the X-ray spectrum is building up almost simultaneously. This digital quantum counting technique makes the energy dispersive spectrometry exceedingly reliable. A semiconductor material is used to detect the X-rays together with processing electronics to analyses the spectrum.

Anti microbial activity of synthesized nanoparticles

The antibacterial activity of the synthesized nanoparticles was studied. Antibacterial activities of the synthesized silver nanoparticles were tested against human pathogenic bacteria viz., *Escherichia coli, Enterococcus faecalis, Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Salmonella typhi, Vibrio cholerae* by disc diffusion method. All microorganisms were obtained from the National Chemical Laboratory (NCL), Pune, India and were maintained at 4ºC on nutrient agar.

Invitro study:

(iv) Cell line maintenance and growth conditions

Hep2 cell line and Vero cell line were purchased from NCCS (National Centre for Cell Sciences) Pune, India. The cell lines were maintained at 37^0C at 5% CO_2 in CO_2 incubator. Cultures were viewed using an inverted microscope to assess the degree of confluency and the absence of bacterial and fungal contaminants were confirmed.

(v) Cytotoxicity test

MTT Cell Proliferation Assay was employed to estimate the cytotoxicity of synthesized nanoparticle. Cells were first transferred (200µL/well at 7.5×104mL) into 96 well plates and incubated for 24 hr. The original media was removed and 100 µL fresh media was added. Synthesized nanoparticle was added at concentration of 20-160 µg and then final volume was made to 200µl with the media and incubated for 4 hr. After incubation media containing drug was removed. MTT reagent 20 µL was added to each well containing media and incubated for 3.5 hr at 37 °C under an atmosphere of 5% CO_2 until a purple precipitate was visible. Media was removed carefully (Do not disturb cells and do not rinse with PBS). 150µl DMSO (MTT solvent) was added to dissolve the purple precipitate. Absorbance was read at 570 nm with a reference filter of 630 nm.

RESULTS AND DISCUSSION

Biosynthesis of silver nanoparticle

Synthesis of silver nanoparticle using stem extract of *Cissus quadrangularis* by microwave irradiation was reported in this study. Microwave irradiation is a rapid and convenient method for the synthesis of nanoparticles [15]. 100ml of 1mM silver nitrate solution containing 10 ml of stem extract was exposed to microwave irradiation. After 5 min exposure to the irradiation the color of the solution was changed from watery to yellowish brown color (fig 1A), due to reduction of silver ion; which indicates the formation of silver nanoparticles. It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles [9]. Further the reduction was confirmed by UV spectrometer analysis.

UV-Vis Spectra analysis

The formation of silver nanoparticles by reduction of the aqueous silver ions during exposure of *Cissus quadrangularis* extract under microwave irradiation may be easily followed by UV-Vis spectroscopy. Silver nanoparticles exhibit brown color in aqueous solution due to the surface Plasmon resonance phenomenon [13,1]. The band observed at UV spectrum corresponding to surface Plasmon resonance occurs at 422nm (fig 1B) and clearly indicates the formation of nanoparticles in the solution as the exact position of absorbance depends on the number factors such as the dielectric constant of medium and size of the particle [17].

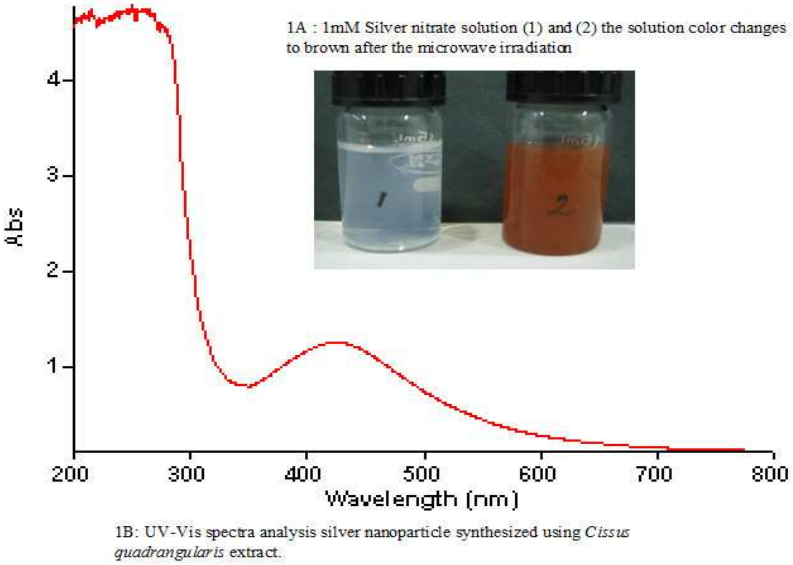

Figure 1A

1mM Silver nitrate solution (1) and (2) the solution color changes to brown after the microwave irradiation.

Figure 1B: UV-Vis spectra analysis silver nanoparticle synthesized using *Cissus quadrangularis* extract.

EDAX analysis

Analysis through Energy dispersive X-ray (EDX) spectrometers confirmed the presence of elemental silver signal of silver nanoparticles (Fig.2) the vertical axis displays the number of X-ray counts whilst the horizontal axis displays energy in K eV. Identification lines for the major emission energies for silver (Ag) are displayed and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified.

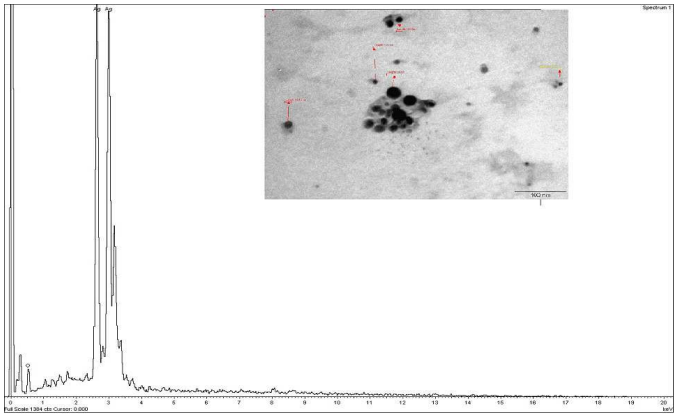

Figure. 2
EDAX and TEM image of silver nanoparticles synthesized using Cissus quadrangularis

TEM analysis

High resolution transmission electron microscope was carried out to visualize the size and shape of the silver nanoparticles. Fig (2) represents the silver nanoparticle, which were synthesized by using *Cissus quadrangularis.* A quick preparation by the deposition of sample containing the silver nanoparticle onto support copper grids or drop coated films HR-TEM revealed the size and shape of the silver nanoparticles. The silver nanoparticles which were synthesized using *Cissus quadrangularis* had dimensions small enough to be electron transparent and imaged as poly-dispersed spherical nanoparticles with variable diameter ranging from 5nm to 30 nm in diameter. The histogram obtained from the enlarged TEM image showed the average nanoparticle size was 15nm in diameter.

Antibacterial activity

The antibacterial activity of silver nanoparticle was tested against the following microorganism by disc diffusion method: *E.coli, Enterococcus faecalis, Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Salmonella typhi, Vibrio cholera* and the results were tabulated in the Table 1. The silver nanoparticle has shown antibacterial activity against all tested microorganism and maximum zone of inhibition was found against *Vibrio cholerae .*

Table 1
Antibacterial activity of silver nanoparticle

Microorganism	zone of inhibition in mm		
	10µl of 1mM $AgNO_3$	10 µl of plant extract	10µl of Ag nanoparticle
Escherichia coli	Nil	Nil	10
Enterococcus faecalis	8	4	13
Bacillus subtilis	Nil	6	13
Klebsiella pneumonia	7	Nil	14
Staphylococcus aureus	Nil	Nil	14
Salmonella typhi	9	7	14
Vibrio cholera	7	8	15

Cytotoxicity (MTT) assay

The Cytotoxicity of the silver nanoparticle was studied In-vitro against Hep2 cell and Vero cell line at different concentration (20, 40, 60, 80, 100, 120, 140, 160µg). The concentration required for 50% cell death (IC_{50}) for HEP2 and Vero cell line were found to be 64µg and 90µg respectively. When compared to Vero cell line, for Hep2 cell line less IC_{50} value was needed (fig 3).

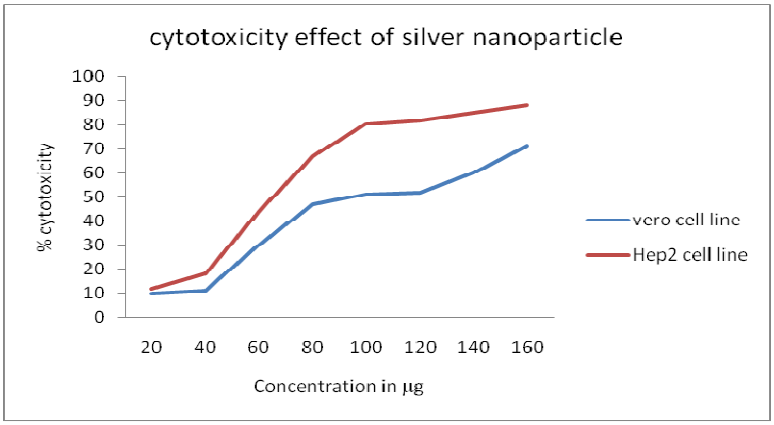

Figure 3
Cytotoxicity effect of silver Nanoparticle on Hep-2 and Vero cell line

DISCUSSION

Biosynthesis of silver nanoparticle : Biosynthesis methods of nanoparticles preparations, employing either biological microorganisms or plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical method [15]. The time required to complete the nanoparticle formation reaction is a slow process, the reaction ranged from 24- 120hr; this lengthy reaction is the one drawback of the biological synthesis. This drawback can be overcome by means of microwave irradiation method, a novel combinatorial synthesis approach which is rapid, simple and green for the synthesis of metallic nanostructures of noble metals such as silver. In this study, the silver nanoparticle was synthesized by a combination of plant extract (stem extract of *Cissus quadrangularis*) and microwave irradiation. The microwave irradiation heats up a material through its dielectric loss, which converts the radiation energy into thermal energy [15]. The advantage of using the microwave irradiation is that it provides uniform heating around the nanoparticle and can assist the digestive ripening of such particle without aggregation. When the silver nitrate solution having the stem extract was treated with microwave irradiation, the color change was observed after 5 min exposure. The solution color was changed from colorless to yellowish color, which indicates the reduction of silver ions to silver nanoparticle.

Characterization of the silver nanoparticle

UV-Vis Spectra analysis: In metal nanoparticle, the conduction band and valence band lie very close to each other and though these electrons move freely. The free electrons give rise to a surface Plasmon resonance band, occurring due to the collective oscillation of electrons of silver nanoparticle in resonance with the light wave. Classically, the electric field of an incoming wave induces polarization of the electrons with respect to much heavier ionic core of nanoparticles [17]. As a result a net change difference occurs, which in turn acts as a restoring force. This creates a dipolar oscillation of all the electromagnetic field becomes resonant with the coherent electron motion; a strong absorption takes place, which

is the origin of the observed color, which was yellowish brown in our observation. This absorption strongly depends on the particle size, dielectric medium and chemical surroundings. The UV- visible spectra recorded for the aqueous solution containing silver nanoparticle, it can be observed that the silver surface Plasmon band occurs at around 422nm.

EDAX analysis: The elemental analysis was done by EDAX. From the EDAX spectrum (fig2) a strong signals from silver atoms and weaker signals from O atoms was observed. The weaker signals were proventients from protein/ enzymes of the stem extract.

TEM analysis: The TEM analysis was employed to visualize the size and shape of the synthesized nanoparticle. TEM image (fig 2) showed the shape of the nanoparticle were spherical in shape and the size ranges from 5-30nm.

Antibacterial activity: Silver has been used for its well known antibacterial properties since roman time however the advances in generating silver nanoparticles have made possible a revival of the use of silver as a powerful bactericide [18]. In this study, antibacterial activity of the silver nanoparticle was tested against the following bacterial culture, *E.coli, Enterococcus faecalis, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi, Vibrio cholerae* and maximum activity of the silver nanoparticle was found to be against *Vibrio cholerae*.

Cytotoxicity (MTT) assay: The cytotoxicity effect of synthesized silver nanoparticle on normal and cancer cell was studied at different concentration (20 µg, 40 µg, 60 µg, 80 µg, 100 µg, 120 µg, 140 µg, 160µg) by MTT assay. The silver nanoparticle was able to reduce the viability in dose dependent manner. After 4 hours of treatment, the silver nanoparticles were found to more toxic to cancer cell than the normal cell. Both the normal and cancer cell line has shown different pattern of dose-dependent cytotoxicity response of silver nanoparticle. For cancer cell line at 20µg concentration the percentage toxicity was 12%. With increase in the dose there was increase in the percentage toxicity and IC_{50} (50 % cytotoxicity) value was found to be 64 µg for cancer cell line. But for the normal cell at 20 µg concentration the percentage toxicity was found to be 10% and IC_{50} value was found to be 90 µg. Compared to normal cell the cancer cell needed less dosage to kill the cell. This preliminary cytotoxicity study of silver nanoparticle might contribute to the comprehensive of this compound in cancer studies.

CONCLUSION

The synthesis, characterization and application of biologically synthesized nanomaterials have become an important branch of nanotechnology. In this study the bio-reduction of aqueous Ag+ ions by the stem extract of the plant *Cissus quadrangularis* was studied. The reduction of the silver ions through stem extracts leading to the formation of silver nanoparticles. A synthesized silver nanoparticle was characterized by UV-Vis spectrophotometer, TEM and EDAX analysis. The antimicrobial activity of silver nanoparticles was studied against bacteria (*E.coli, Enterococcus faecalis, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi, Vibrio cholerae).* Maximum antibacterial activity was observed against *Vibrio cholerae,* when compared to other bacteria. Anticancer activity and cytotoxicity of *Cissus quadrangularis* synthesized silver nanoparticles was studied by MTT assay. IC 50 value for the Hep2 and Vero cell line was found to be 64µg and 90µg respectively. This green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability etc. Applications of such eco-friendly

nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). Toxicity studies of silver nanoparticles on human pathogen and on cancer cell opens a door for a new range of antibacterial agents and anticancer agents.

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